a) BLC = RP-II protease from *Bacillus licheniformis*,

b) AA513 = RP-II protease from *Bacillus halmapalus* AA513

c) AC116 = RP-II protease from *Bacillus licheniformis* AC116

d) BO32 = RP-II protease from *Bacillus pumilus* BO32

e) CDJ31 = RP-II protease from *Bacillus licheniformis* CDJ31

f) JA96 = RP-II protease from *Bacillus pumilus* JA96

g) MPR = RP-II protease from *Bacillus subtilis* IS75

```
a)   1 SVIGSDDRTRVTNTTAYPYRAIVHIS....SSIGSCTGWMIGPKTVATAG 46
b)     VVIGDDGRRQVQNTSFMPFRALTYIEFGNLTSTWSCSGGVIGTDLVVTNA

a)  47 HCIYDTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGWR.SGNTNY 95
b)     HCV....EGSVL.AGTVVPGMNNSQWAYGHYRVTQIIYPDQYRNNGASEF

a)  96 DYGAIELS.....EPIGNTVGYFGYSYTTSSLVGTTVTISGYPGDKTAGT 140
b)     DYAILRVAPDSDGRHIGNRAGILSFTETGTVNENTFLRTYGYPGDKISET

a) 141 Q....WQHSG.PIAISETYKLQYAMDTYGGQSGSPVFEQSSSRTNCSGPC 185
b)     KLISLWGMVGRSDAFLHRDLLFYNMDTYFGQSGSPVLNSVDSMVAVHNAG

a) 186 SLAVHTNGVYGGSSYNRGTRITKEVFDNLTNWKNSAQ 222
b)     YIVGGNREINGGPKIRRDFTNLFNQMN.........

a)   1 SVIGSDDRTRVTNTTAYPYRAIVHISSSIGSCTGWMIGPKTVATAGHCIY 50
c)     SVIGSDERTRVTDTTAFPYRAIVHISSSIGSCTGWLIGPKTVATAGHCVY

a)  51 DTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGWRSGNTNYDYGAI 100
c)     DTASRSFAGTATVSPGRNGSAYPYGSVTSTRYFIPSGWQSGNSNYDYAAI

a) 101 ELSEPIGNTVGYFGYSYTTSSLVGTTVTISGYPGDKTAGTQWQHSGPIAI 150
c)     ELSQPIGNTVGYFGYSYTASSLAGAGVTISGYPGDKTTGTQWQMSGTIAV

a) 151 SETYKLQYAMDTYGGQSGSPVFEQSSSRTNCSGPCSLAVHTNGVYGGSSY 200
c)     SETYKLQYAIDTYGGQSGSPVYEKSSSRTNCSGPCSLAVHTNGVYGGSSY

a) 201 NRGTRITKEVFDNLTNWKNSAQ 222
c)     NRGTRITKEVFDNFTSWKNSAQ 222
```

Fig. 1a

```
a)   1 SVIGSDDRTRVTNTTAYPYRAIVHISSSIGSCTGWMIGPKTVATAGHCIY 50
d)     VVIGDDGRTKVANTRVAPYNSIAYTTFGGSSCTGTLIAPNKILTNGHCVY

a)  51 DTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGW.RSGNTNYDYGA 99
d)     NTASRSYSAKGSVYPGMNDSTAVNGSANMTEFYVPSGYINTGASQYDFAV

a) 100 IELSEPIGNTVGYFGYSYTTSSLVGTTVTISGYPGDKTAGT....QWQHS 145
d)     IKTDTNIGNTVGYRSIRQVT.NLTGTTIKISGYPGDKMRSTGKISQWEMS

a) 146 GPIAISETYKLQYAMDTYGGQSGSPVFEQSSSRTNC.SGPCSLAVHTNGV 194
d)     GPVTREDTNLAYYMIDTFSGNSGSAMLDQNQQIVGVHNAGYSNGTINGGP

a) 195 YGGSSYNRGTRITKEVFDNLTNWKNSAQ 222
d)     KATAAFVEFINYAKAQ.............
```

```
a)   1 SVIGSDDRTRVTNTTAYPYRAIVHISSSIGSCTGWMIGPKTVATAGHCIY 50
e)     SVIGSDERTRVTNTTAYPYRAIVHISSSIGSCTGSLIGPKTVATAGHCIY

a)  51 DTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGWRSGNTNYDYGAI 100
e)     DTASGSFAGTATVSPGRNGSTYPYGSVTSTRYFIPSGYRSGNSNYDYGAI

a) 101 ELSEPIGNTVGYFGYSYTTSSLVGTTVTISGYPGDKTAGTQWQHSGPIAI 150
e)     ELSQPIGNTVGYFGYSYTTSSLVGSSVTIIGYPGDKTSGTQWQMSGNIAV

a) 151 SETYKLQYAMDTYGGQSGSPVFEQSSSRTNCSGPCSLAVHTNGVYGGSSY 200
e)     SETYKLQYAIDTYGGQSGSPVYEASSSRTNCSGPCSLAVHTNGVYGGSSY

a) 201 NRGTRITKEVFDNLTNWKNSAQ 222
e)     NRGTRITKEVFDNLTNWKNSAQ
```

```
a)   1 SVIGSDDRTRVTNTTAYPYRAIVHISSSIGSCTGWMIGPKTVATAGHCIY 50
f)     VVIGDDGRTKVTNTRVAPYNSIAYITFGGSSCTGTLIAPNKILTNGHCVY

a)  51 DTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGW.RSGNTNYDYGA 99
f)     NTATRSYSAKGSVYPGMNDSTAVNGSANMTEFYVPSGYINTGASQYDFAV

a) 100 IELSEPIGNTVGYFGYSYTTSSLVGTTVTISGYPGDKTAGT....QWQHS 145
f)     IKTDTNIGNTVGYRSIRQVT.NLTGTTIKISGYPGDKMRSTGKVSQWEMS

a) 146 GPIAISETYKLQYAMDTYGGQSGSPVFEQSSSRTNC.SGPCSLAVHTNGV 194
f)     GPVTREDTNLAYYTIDTFSGNSGSAMLDQNQQIVGVHNAGYSNGTINGGP

a) 195 YGGSSYNRGTRITKEVFDNLTNWKNSAQ 222
f)     KATAAFVEFINYAKAQ.............
```

Fig. 1b

```
                      .         .         .         .
a)     1 SVIGSDDRTRVTNTTAYPYRAIVHIS......SSIGSCTGWMIGPKTVAT 44
g)       SIIGTDERTRISSTTSFPYRATVQLSIKYPNTSSTYGCTGFLVNPNTVVT

                  .         .         .         .         .
a)    45 AGHCIYDTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGW.RSGNT 93
g)       AGHCVYSQDHG.WASTITAAPGRNGSSYPYGTYSGTMFYSVKGWTESKDT

                   .         .         .         .         .
a)    94 NYDYGAIELSEPIGNTVGYFGYSYT.TSSLVGTTVTISGYPGDKTAGTQW 142
g)       NYDYGAIKLNGSPGNTVGWYGYRTTNSSSPVGLSSSVTGFPCDKTFGTMW

                   .         .         .         .         .
a)   143 QHSGPIAISETYKLQYAMDTYGGQSGSPVFEQSSSRTNCSGPCSLAVHTN 192
g)       SDTKPIRSAETYKLTYTTDTYGCQSGSPVYRNYSD....TGQTAIAIHTN

                   .         .         .
a)   193 GVYGGSSYNRGTRITKEVFDNLTNWKNSAQ 222
g)       ...GGSSYNLGTRVTNDVFNNIQYWANQ..
```

Fig. 1c

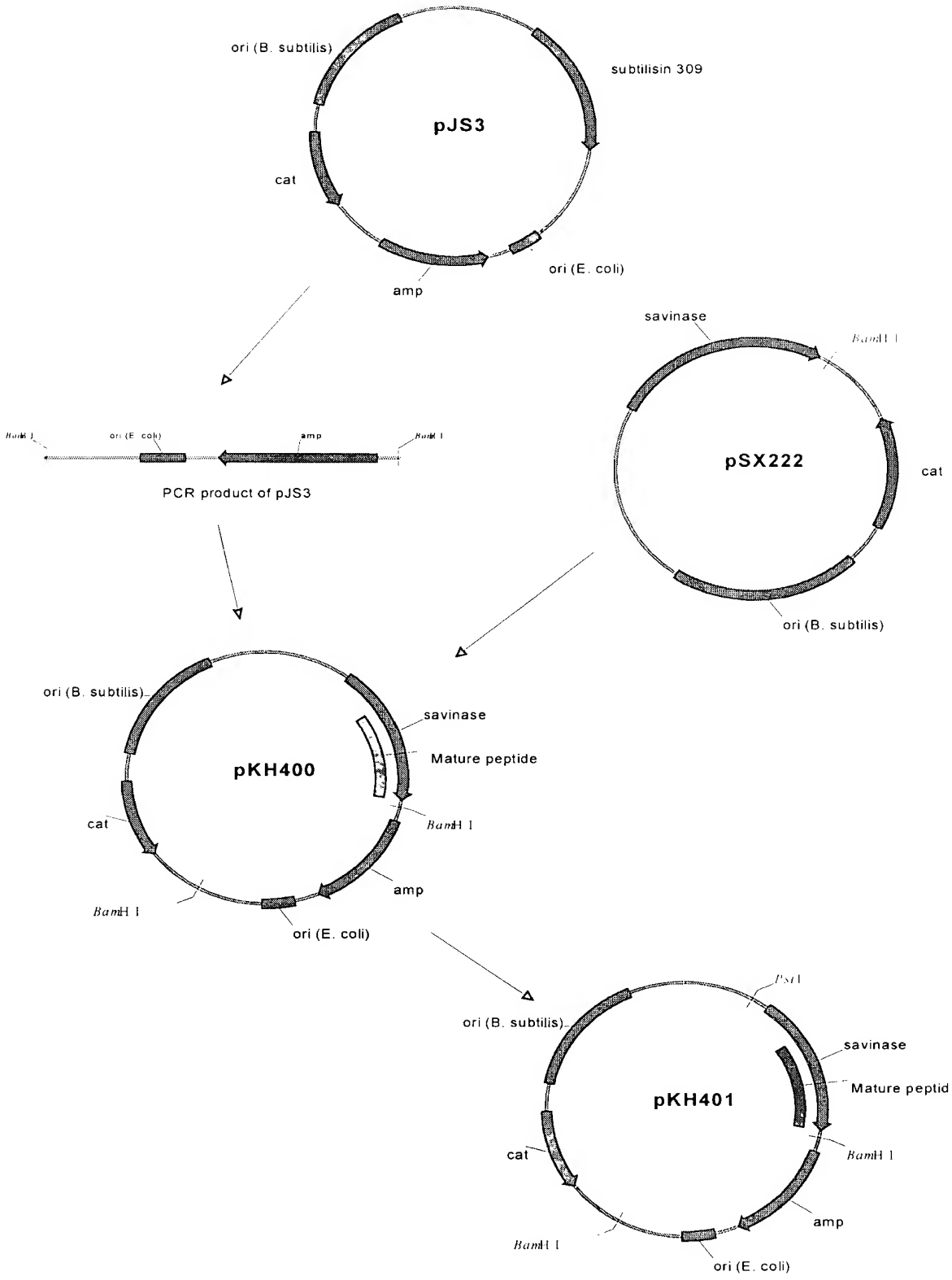
ori (B. subtilis)
subtilisin 309
pJS3
cat
ori (E. coli)
amp
savinase
BamH I
pSX222
cat
ori (B. subtilis)
BamH I
ori (E. coli)
amp
BamH I
PCR product of pJS3
ori (B. subtilis)
savinase
Mature peptide
pKH400
cat
BamH I
amp
BamH I
ori (E. coli)
PstI
ori (B. subtilis)
savinase
Mature peptid
pKH401
cat
BamH I
amp
BamH I
ori (E. coli)

Fig 2a

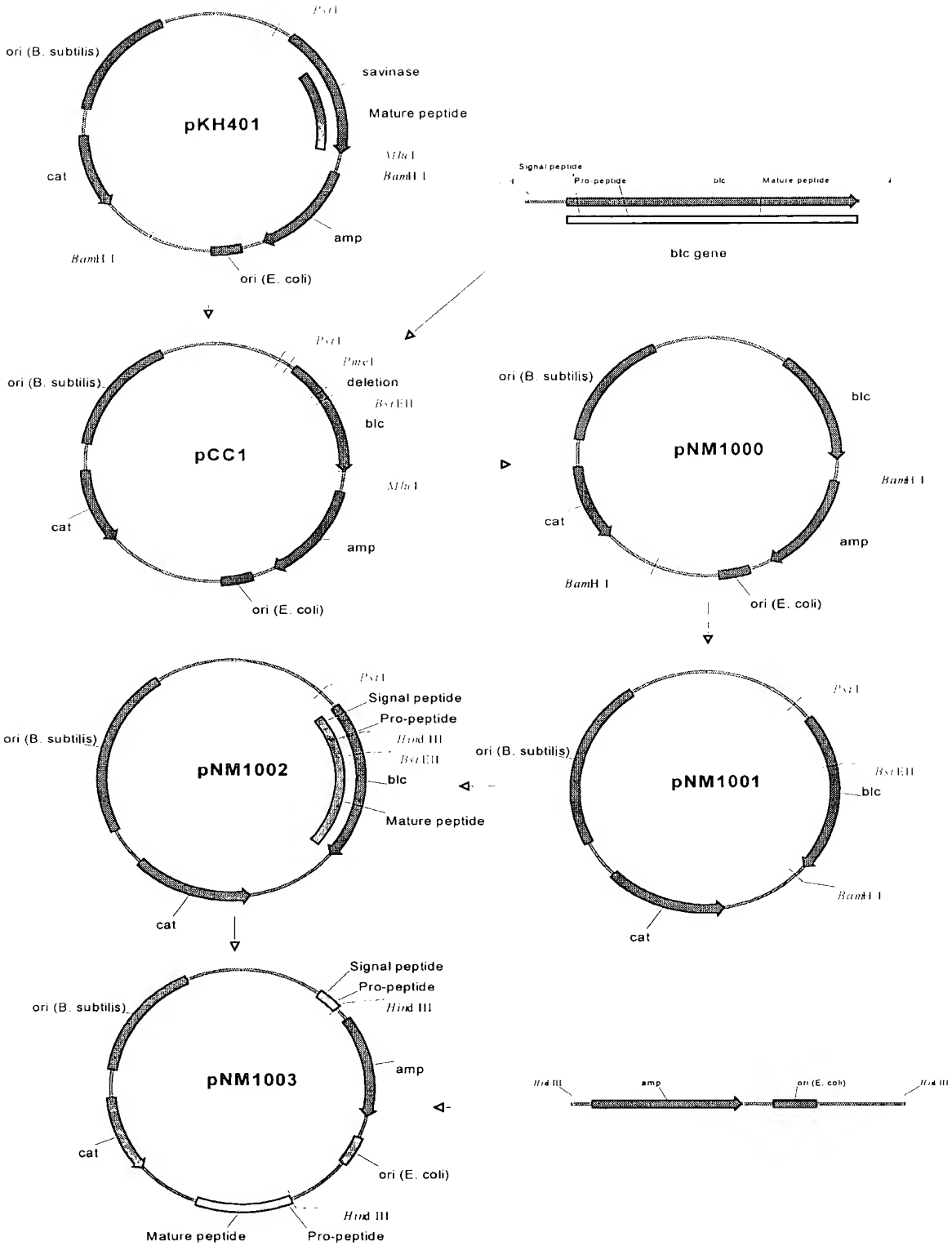
pKH401
ori (B. subtilis)
savinase
Mature peptide
cat
amp
ori (E. coli)
Signal peptide
Pro-peptide
blc
Mature peptide
blc gene
pCC1
deletion
blc
cat
amp
ori (E. coli)
pNM1000
ori (B. subtilis)
blc
cat
amp
ori (E. coli)
pNM1002
Signal peptide
Pro-peptide
blc
Mature peptide
cat
pNM1001
ori (B. subtilis)
blc
cat
pNM1003
Signal peptide
Pro-peptide
ori (B. subtilis)
amp
cat
ori (E. coli)
Mature peptide
Pro-peptide
amp
ori (E. coli)

Fig 2b